The Possible Human Hazard of the Naturally Occurring Bracken Carcinogen

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Work in Bangor demonstrated conclusively that the bracken fern (*Pteridium aquilinum*) contains a potent carcinogen for rats, producing malignant intestinal adenocarcinoma in nearly all animals (Evans & Mason, 1965; Mason, 1965). Since then similar results have been obtained with purified bracken extracts, and strong carcinogenic activity has been demonstrated in other species including mice, quail and guinea pigs (Widdop, 1967; Barber, 1969; Evans, 1968, 1970; Evans, Widdop & Barber, 1967; Evans, Barber, Jones & Leach, 1968). The target organs vary with the species but include stomach, small intestine, caecum, colon, lung, urinary bladder and the reticulo-endothelial system. In some cases single oral or intraperitoneal doses of purified extract are sufficient.

Turkish and American workers have reported the experimental production of urinary-bladder tumours in cattle and the presence of a carcinogen in the urine (Pamukcu, Göksoy & Price, 1967; Pamukcu, Olson & Price, 1966).

The possible human hazard could be by direct consumption of bracken as occurs notably in Japan, or indirectly by such pathways as milk and dairy products, meat or the water supply. Workers in Japan have shown that even after culinary preparation the bracken is still strongly carcinogenic for rats, both the intestine and bladder being involved (Hirono, Shibuya, Fushimi & Haga, 1970). The Japanese canned bracken, as tested here by extraction and the bacteriophage reversion assay, has given a positive result (Leach, 1970).

The indirect consumption through the milk could be even more important, since this would not be limited to such a short season, and, further, we have demonstrated an age-dependence in rats, where it is the young animal that is at risk (Evans & Widdop, 1966). It would extend the hazard beyond Japan to any region where grazing animals can and do ingest the fern, especially under free range and upland marginal farming conditions.

We have already demonstrated in mice the maternal transfer of the carcinogen to the offspring via the placenta and/or milk (R. S. Jones, unpublished work; Evans, Barber, Jones & Leach, 1969). Although direct chemical tests for the presence of the toxin in bovine milk give positive results, this does not necessarily mean that it still has biological activity; also, different active metabolites may have been formed in the cow. We are therefore currently testing the milk from cows receiving a bracken supplement for long-term carcinogenic action by feeding both the whole and processed milk to rats and mice.

While waiting for these results we have fed the same milk to a young calf and recorded the peripheral blood counts. These have definitely shown that the bone marrow of the calf responded by impairment of activity, as it does in the directly produced acute bracken poisoning.

The significance of this finding depends on the common identity of the acute cattle toxin and the carcinogen. At present, practical difficulties do not allow testing of the post-chromatography toxin on cattle, but we have twice shown that purified extracts before this final separation have the capacity to produce both syndromes. This has been done by using a series of solvent extractions with calves, rats and mice (Widdop, 1967; Evans, Widdop & Barber, 1967). There were also indications of mutagenic activity (Barber, 1969). Later, in work with another extraction procedure involving charcoal and ethyl acetate precipitation, the final extract was positive for carcinogenic, mutagenic and calf activity (Evans, Barber, Jones & Leach, 1969; D. L., Jones, unpublished work).

In conclusion, it may be mentioned that there is high rate of human stomach cancer with obscure aetiology prevalent in Japan and in North Wales, and also that the world rate as a whole is declining. If it is possible that bracken plays any part at all, perhaps this decline would reflect gradual improvement in agricultural practices as well as nutritional standards.

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Phenolic Metabolism in Potato Tuber Tissue after Infection with *Phytophthora infestans*

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Phenolic compounds accumulate in potato tissue after inoculation with *Phytophthora infestans*. Chlorogenic acid, scopolein (Hughes & Swain, 1960) and a lignin-like polymer (Friend & Knee, 1969) have been found in infected susceptible tubers (var. Majestic), whereas suspended tissue cultures of R_1-resistant potatoes (var. Orion) accumulate salicylic acid, vanillic acid and *p*-hydroxybenzoic acid after inoculation with an incompatible race of the fungus (Robertson *et al.* 1968).

We compared the changes in phenolic compounds and of phenylalanine ammonia-lyase activity of tubers of susceptible and resistant varieties after inoculation with a sporangial suspension of *P. infestans*. There was a large increase in chlorogenic acid concentration in both inoculated and control discs of Orion (resistant) compared with similarly treated Majestic (susceptible) tubers. Phenylalanine ammonia-lyase activity also increased more rapidly in Orion than in Majestic tubers, and this higher activity was maintained. There was a greater accumulation of a lignin-like polymer in both sets of inoculated tubers; the increase in Orion was more than in Majestic tubers.

The changes in lignification in relation to the spread of the fungus across the surface of tuber slices from a central inoculation site were also examined. In Majestic tubers the infected zone was 50mm in diameter after 140h, but in Orion tubers it did not extend beyond 15mm, a distance reached in 40h. In Orion tubers maximum lignification was after 70h near the centre of the infected area, whereas in Majestic tubers lignification was spread over a wider area and the highest values were obtained in the outer portion of the infected area after 140h. The values of phenylalanine ammonia-lyase activity followed a similar pattern.

Lignification may well be involved in the restriction of the spread of the fungus in Orion tubers; similar suggestions have been made for other host–parasite combinations (Hijwegen, 1963) and the extent of lignification could be controlled by the activity of phenylalanine ammonia-lyase. Lignin could act as a barrier by modifying the galactan-containing polymers in the cell wall so that they can no longer be hydrolysed by the *P. infestans* galactanase (Knee & Friend, 1970).

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The Effect of γ-Irradiation on the Amino Acid Content of Proteins

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The technology of γ-irradiation has been developed and applied to foods to prevent loss by insect depredation and by bacterial and fungal attack. The hazards of radiation have led to intensive research applied to the task of showing that irradiated foods are safe to eat. Far less effort has gone into a study of the effect of radiation on the nutrients contained in the foods. There is evidence that radiation can cause change or destruction of free amino acids. Amino acids in proteins are also susceptible, but reports vary as to the nature and extent of the damage. The discrepancies in the literature may result from variations in the conditions of irradiation, such as temperature, oxygen partial pressure, water content, dose and dose rate.

We have investigated the amino acid content of accelerated-freeze-dried egg (Affined Foods Ltd., High Wycombe, Bucks., U.K.) and two batches of wheat gluten (BDH Chemicals Ltd., Poole, Dorset, U.K.). The moisture contents were 1.6, 6 and 10% respectively. These were distributed into cellophan–polythene laminate bags, sealed under 96% N_2, and irradiated at room temperature at dose rates of approx. 1.5Mrd/h in the Spent Fuel Rod Assembly, Atomic Energy Research Establishment, Harwell, Berks, U.K., for total doses of 0.0, 0.5, 2.5 and 5.0Mrd, and, for the second batch of gluten, 10Mrd. After irradiation and transport to our laboratory, the samples were stored at −20°C until analysed by hydrolysis and ion-exchange chromatography. In egg the only amino acids significantly damaged were methionine and tryptophan. In gluten tryptophan in both batches, and threonine in batch 1 and methionine in batch 2 were significantly diminished. An apparent effect on aspartic acid, serine, glutamic acid, glycine, leucine, tyrosine